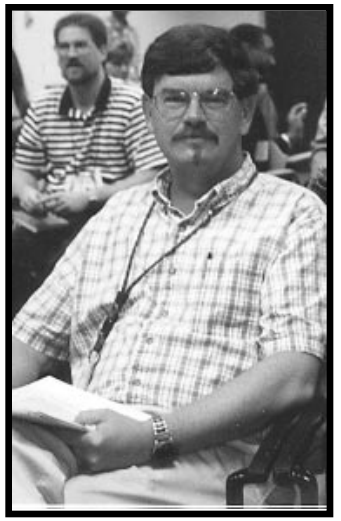
HIV IMMUNOLOGY and DIAGNOSTICS



HIV Immunology and Diagnostics Branch

Introduction

The HIV Immunology and Diagnostics Branch, DASTLR, conducts basic and applied studies of microbial-host interactions that occur in infections, particularly infection with HIV. Studies are conducted on diagnostics, natural history, mechanisms of infection, immunopathogenesis, and the biology of host-microbe interaction. These studies are conducted to improve diagnostic accuracy, to analyze and distinguish effective versus deleterious immune responses, and to identify targets for immune intervention. Branch activities involve a) developing, evaluating, and improving assay procedures for immune mechanisms and diagnosis of diseases; b) performing diagnostic testing for laboratories and organizations within NCID and CDC as well as outside organizations; and c) performing or collaborating in the performance of clinical, epidemiologic, and field studies of immunologic disease states. The Branch comprises four sections: the Cellular Immunology Section, the Immunogenetics Section, the Developmental Technology Section, and the Serology Section.

1998 Accomplishments

Immunobiology of HIV Infection

HIV coreceptor expression. Continued studies to examine the expression of HIV coreceptors, CXCR4 and CCR5, on lymphocyte subpopulations in HIV-positive and -negative individuals.

Clinical flow cytometry quality control. Completed studies examining the use of fluorescent particles to standardize fluorescence measurements on flow cytometers. Examined properties of a stabilized, whole-blood control for immunophenotyping quality control. Prepared manuscript describing and summarizing these experiments.

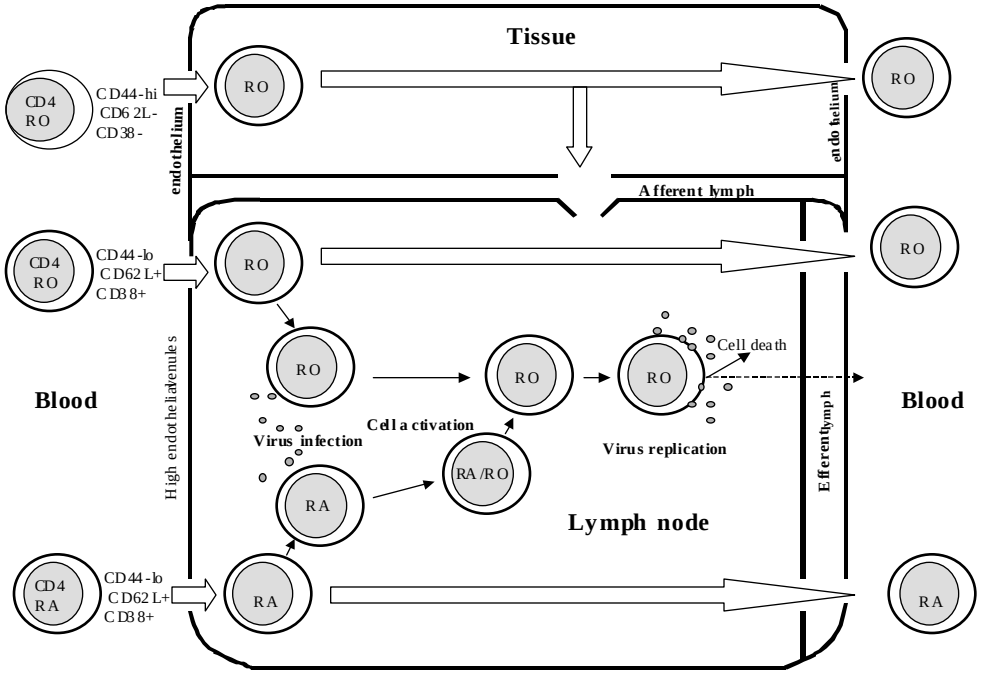
Chemokine effects on the immune response. Examined the effects of selected chemokines on a normal immune response.

Activation of lymphocytes. Optimized assays for Ca++ influx with human peripheral blood lymphocytes in response to chemokines.

Cytokines and motality from infectious disease. As part of a study of HIV infection in hospitalized Thai patients, determined that production of TNF- by CD3+CD16/56+ lymphocytes was inversely associated with mortality in HIV-infected patients, independent of the presence or absence of associated opportunistic infections (OI). A manuscript describing these findings was published in January 1999.

HIV infection in vitro. Published manuscript concerning activation markers and the increased expression of CD80 and CD86 on CD3+ cells producing cytoplasmic HIV-1 p24; completed manuscript concerning the effects of HIV infection and antigens on CD3, CD4, and CD8; began preparing manuscript concerning the modulatory effects of anti-CD3 and mitogens on CD3, CD4, and CD8.

Kawasaki disease. Published manuscript concerning Kawasaki disease and the T-cell antigen receptor (TCR).



CD4 T-cell recirculation and HIV infection

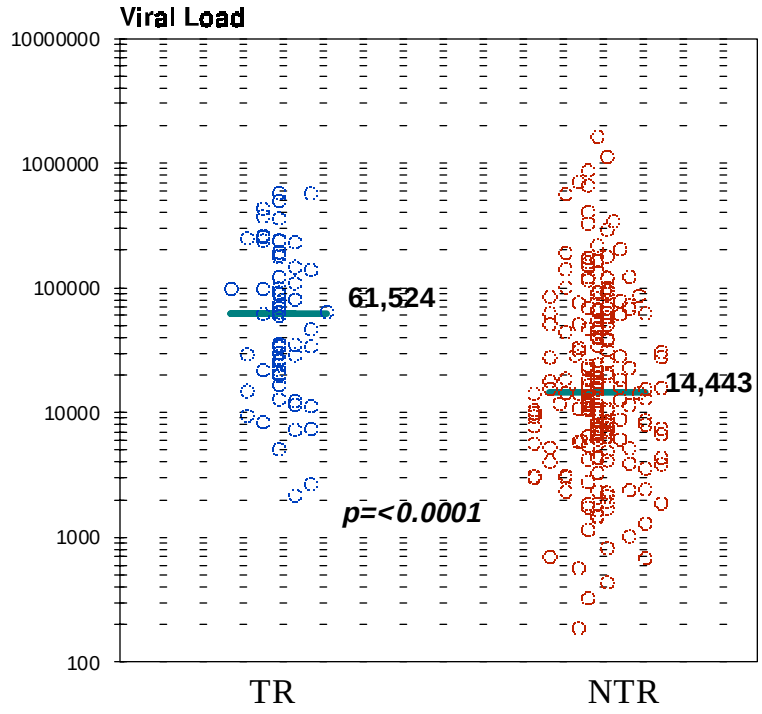
HIV entry mechanisms. Improved assay for tracking HIV entry by subcellular fractionation and immunoprecipitation. As part of these studies, prepared antisera to HIV chemokine coreceptors (CXCR4 and CCR5). Improved monitoring of cell surface structures during HIV infection.

CD4 T-cell turnover. Completed study of CD4 T-cell regeneration in patients undergoing triple-drug antiretroviral therapy. One manuscript is in press and another has been submitted.

Maternal-child transmission. In collaboration with the Division of HIV/AIDS Prevention—Surveillance and Epidemiology (DHAP-SE)/NCHSTP, completed studies of viral load, CD4 T-cell levels, and other maternal factors at delivery and their relationship to perinatal HIV transmission. A manuscript describing results has been published.

T-cell activation and viral load. Completed a serial study of viral load determinations and "immune-activating" events in 18 individuals who were clinically followed for 26 weeks to assess the effect of immune-activating events on viral load. A manuscript describing results is in preparation.

Maternal Viral Load and Perinatal Transmission
Thai Cohort



Needlestick study. Collaborated with investigators in the HIV Infection Branch, Hospital Infections Program (HIP), NCID, in a multicenter study of health-care workers (HCWs) exposed to HIV to determine whether any immunologic response to HIV occurs following such exposures. Coordinated the laboratory testing for this study. Because the number of HIV-related needlesticks occurring was lower than expected, the study was extended for an additional year.

ICL. Conducted ongoing longitudinal study of idiopathic CD4+ T-lymphocytopenia (ICL). Provided consultation to physicians of ICL patients in the United States. In contrast to results presented by other investigators, found no association of ICL and human herpesvirus 8 (HHV-8) infection.

HIV clinical course.

Initiated a collaborative study with the Veteran's Affairs Medical Center (VAMC) and HIP/NCID to longitudinally assess the relationships among intracellular cytokine patterns, HIV viral titers, CD4 cell counts, and other immune markers on the clinical course of HIV infection both before and during antiretroviral therapy. A pilot study to assess rapid changes post initiation of therapy and to determine optimal time intervals for further assessments began in February 1999.

Collaborated with investigators in the HIV/AIDS and Retrovirology Branch, DASTLR, to study prognostic markers for HIV progression. Published a paper on the finding that adaptation to promiscuous usage of CC and CXC-chemokine coreceptors *in vivo* correlates with HIV-1 progression.

Also in collaboration with investigators in the HIV/AIDS and Retrovirology Branch, and with investigators at the Retroviral Genetics Laboratory, Center for Virus Research, Westmead Institutes for Health Research, Westmead, NSW, Sydney, Australia, studied the evolution of HIV-1 quasispecies in a longitudinal cohort of American patients with different disease progression rates; findings indicated that the extended V2 region with acquisition of the N-linked glycosylation site was uniquely present in slow and long-term nonprogressors (manuscript submitted).

Continued a study of HIV-associated lymphadenopathy in a cohort of homosexual men. This cohort study, which began in 1982, has examined prognostic factors for HIV progression.

HHV-8

Collaborated with the Viral Exanthems and Herpesvirus Branch, Division of Viral and Rickettsial Diseases (DVRD)/ NCID, and investigators at Emory University School of Medicine in studies of a new herpesvirus (HHV-8) associated with Kaposi's sarcoma.

Participated in the evaluation of serologic and PCR-based tests for the virus (results presented at HHV-8 conference; manuscript in preparation).

Began a retrospective study of infection with HHV-8 in a cohort of HIV-infected men who have sex with men in Atlanta.

Participated in the evaluation of genotypes of HHV-8/Kaposi's sarcoma-associated herpesvirus in individuals from North America, Australia, and Africa (manuscript submitted).

Continued efforts to map antigenic domains of HHV-8 and develop assays for HHV-8 antibody detection. Continued to support DVRD's herpesvirus group in developing reagents (antigens and antibodies) for HHV-8 antibody detection.

HIV Immune Response

HIV superantigens. Examined the HIV LTR region known to be homologous to a murine superantigen (SAG) and a highly T-cell immunogenic region of the HIV envelope region for potential SAG activity. (Abstract to be presented at the ASM spring 1999 meetings; manuscript also in preparation).

Cytokines/field studies. In the Malawi study described above, developed and applied methods to conduct T-cell stimulation assays in the field (with minimal laboratory facilities/personnel), transport samples to a central facility (CDC) for cytometric assessment, and maintain fixed cells for up to 6 months.

Cytotoxic T-cell (CTL) studies in HIV-infected persons. Prepared manuscript on new HIV CTL epitopes in Thai HIV subtype E -infected female sex workers (FSWs) in Chiang Rai, Thailand (DHAP-SE/NCHSTP study). Developed alternative CTL protocols suitable for large sample size populations. Initiated new CTL studies on additional HIV-infected persons from Thailand (NCCDHP study). Initiated studies with Abidjan site (DHAP-SE/NCHSTP) to study CTLs in HIV-infected persons. Proposed CTL studies in the Bangkok Municipal Authority (BMA) seroconvertor study (DHAP-SE/NCHSTP).

CTL training. Provided CTL training to researcher from Thai NIH. This person has now established successful HIV CTL assays in Thai NIH and will do more CTL work on persons who are highly HIV-exposed but persistently seronegative (HEPS) and HIV-infected persons in 1999.

CTL studies in HIV-exposed, transiently infected or apparently resistant persons. Prepared manuscript describing the finding of CTL reactivity in HEPS FSWs from Chiang Rai, Thailand. Initiated CTL studies in apparently resistant women from discordant couples in Chiang Mai (NCCDPHP study). Initiated CTL studies of persons with varying HIV exposures in the HIP domestic HCW study; one HCW was found to have a temporal decline in CTL activity following HIV exposure, and we are intensely analyzing this sample for development of a proposed *MMWR* article on HIV-exposed HCWs. Initiated studies with the DHAP-SE/NCHSTP Abidjan site to study CTLs in HEPS individuals.

HIV vaccine studies. Published the first manuscript on HIV vaccine studies in mice. We are continuing to collaborate with Yerkes Primate Research Center on cellular immunology and CTL studies as part of a study of DNA HIV vaccine funded by the National Institutes of Health (NIH). Initiated a new CDC study in macaques on DNA HIV vaccines to complement the Yerkes trial. The DNA vaccine trial is expected to begin at both sites in early 1999.

Human T-lymphotrophic virus (HTLV) studies. Completed the serologic component of a multi-center study to understand the mechanism of reactivity with HTLV proteins of sera from autoimmune disease patients. No increased reactivity to p21e in autoimmune disease patients was observed. Molecular studies of tax genes as well as a manuscript describing findings are in progress.

Perinatal HIV transmission. Published a study of neutralizing antibodies in maternal-child HIV transmission.

CD4 antiviral constructs. Prepared construct of CD4 and C3d (fragment of 3rd component of complement) for use as a potential HIV immunopotentiating agent. Improved expression system.

HIV Drug-Resistance Studies

AZT and maternal child transmission. Using Affymetrix GeneChip technology, completed sequencing of available samples from the pediatric AIDS clinical trials study (PACTS).

New infections in the United States. Completed analysis of available samples from the seroconverter study by automated sequencing, Affymetrix, and Murex Line Probe point-mutation assay. Decided to use automated sequencing for future phases of this study. A manuscript describing these findings is in preparation.

Quality control. Participated in a viral quality assurance program for evaluating HIV-1 drug- resistance genotyping technology.

Occupational exposure among HCWs. In collaboration with HIP, continued to sequence both source patients and HCWs potentially exposed to HIV for HIV-1 drug-resistant mutations and viral relatedness.

Resistence database. In collaboration with the International Activity Branch, DHAP-SE/NCHSTP, developed a database capable of easily tracking samples and data in a linked manner. Developed software that can scan text files to rapidly identify drug-resistance markers.

HIV Diagnostics and Evaluation

Multiplex cytokine assessment. In collaboration with the Scientific Resources Program (SRP)/NCID and with funding from DASTLR, initiated an assessment of multiplex bead technology for determining serum/plasma cytokine levels, providing real-time data analysis, and requiring minimal amounts of samples and reagents. SRP is currently able to perform a five-plex assay (IL-4, -6, -8, -10, and -12) using human serum/plasma.

Cytokine mRNA studies: Published a new sensitive method to detect cytokine mRNA in activated peripheral blood mononuclear cells (PBMCs). Using this assay, we determined that some oil or oil-derived compounds cause cytokine induction in human PBMCs, which may in part explain the pathogenesis of the toxic oil or eosinophilia-myalgia syndrome (TOS/EMS). A manuscript describing these findings is in preparation.

AF-CDC-WHO antinuclear antibody reference lab. Continued to distribute standards; completed and published a multicenter evaluation of ANA testing in normal adults; and completed and published a comparative study of newer ANA testing technologies.

HIV subtyping by sequencing. Evaluated Visible Genetics system for sequencing the C2V3 region of HIV envelope gene.

RT-PCR technology. Evaluated various commercially available sample-preparation technologies for extraction of HIV-1 RNA from serum/plasma. A manuscript describing results is in preparation.

HIV serotyping. Continued to provide HIV serotyping support in Thailand and London. Transferred this serotyping technology to the New York City Department of Health, Roche Molecular Systems, and South Africa. A manuscript from the London group has been accepted for publication in the Journal of Clinical Microbiology.

Serology database. Implemented a computerized, Excel spreadsheet-based system to monitor and retrieve results for routine retrovirus testing. We expect marked improvement in reporting and monitoring efficiency.

Detuned assay.

Produced calibration standards for use in widespread implementation of the detuned assay and completed significant evaluation of the performance characteristics of the assay.

Trained personnel from 10 laboratories in the United States and Canada in performing this assay.

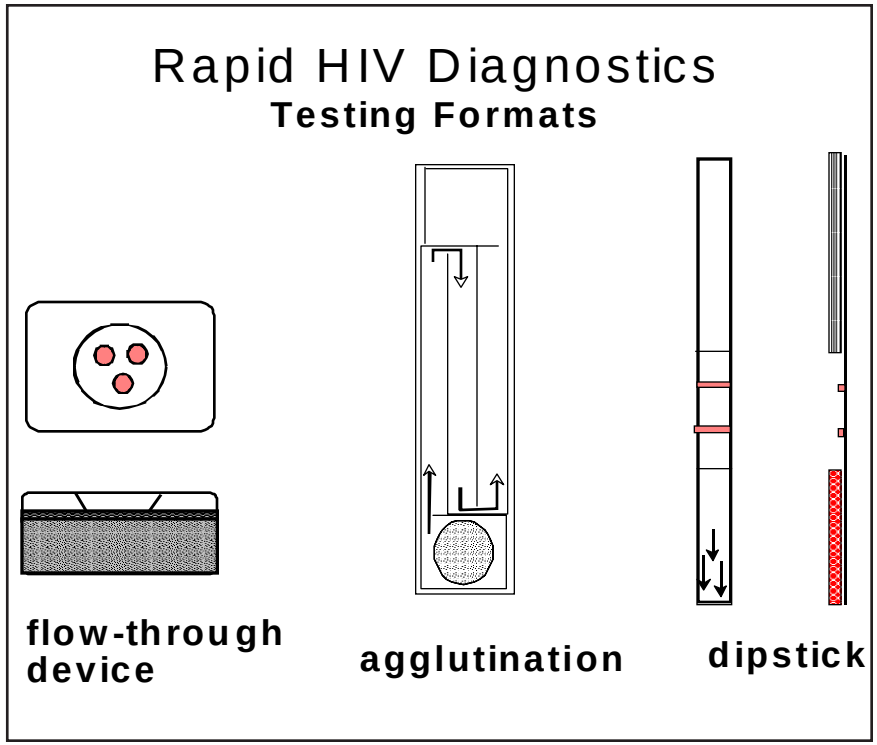
Vaccine studies. Using four different peptide-based EIA tests, tested 297 specimens from vaccine recipients to determine if any of these tests can be used to distinguish vaccination from true infection.

HIV RNA tests. Compared various viral load tests on B and non-B clade HIV strains, and published manuscript on results.

Seroincidence. Completed evaluation of 13 serologic assays based on measurement of antibody quantity, affinity, or isotype in order to characterize an immune response that distinguishes early from established infection.

Unusual HIV cases. Provided consultation to physicians, laboratorians, and patients with unusual exposures to potential HIV infection.

Rapid tests. Completed field-site evaluation of rapid testing technology for HIV antibodies. Initiated evaluation of whole blood, rapid testing methods in preparation for U.S. field studies. Provided supporting data at several conferences (e.g., APHL) indicating that rapid testing algorithms perform equivalently to the standard algorithm.



Immunogenetics

Population studies

Found no evidence indicating a role of CCR5 in HIV transmission susceptibility in a cluster of epidemiologically related HIV-infected persons from upstate New York.

Published finding of a new (IL-10) susceptibility gene for rheumatoid arthritis (RA) in African Americans. Continued studies of other genes in African Americans with RA.

Described a new single nucleotide polymorphisms (SNP) in the IL-6 promotor in African Americans.

Described new recombinant break points in the HLA region of a multicase family with juvenile arthritis.

Continued a collaborative study with the Food and Drug Administration (FDA) to determine if there is a genetic predisposition to developing EMS.

Continued to develop algorithms to study the structure and function of HLA molecules. Continued NIH-collaborative grant with the University of Alabama to apply these algorithms to understanding HLA associations with HIV outcomes in the multicenter AIDS cohort study (MACS) and found some molecular explanations for HLA-A allele associations with progression to AIDS.

Initiated an HLA study of genetic susceptibility to RA after exposure to hepatitis B vaccine.

Initiated studies of HLA susceptibility to tuberculosis and leptopirosis.

HLA genotyping. Performed HLA class I genotyping on 400 specimens from DHAP-SE/NCHSTP epidemiologic studies.

Chemokine receptor polymorphisms. Genotyped for the 32 deletion mutation and sequenced the promoter region of the CCR5 gene on 46 CONRAD specimens,

Gene discovery. Began studies for isolating genes involved in the natural history of HIV infection or required for HIV replication

International

Resistance in non-B subtypes. Adapted amplification and sequencing protocols to non-B subtypes (subtype A from Côte d'Ivoire; subtypes B and E from Thailand) and compared results to those obtained using Affymetrix and Murex.

England. Initiated collaborative study with Imperial College School of Medicine at St. Mary's in London to follow drug resistance in patients infected with non-B subtypes who are receiving triple-drug therapy.

Malawi. Completed the field work for three phases of the collaborative study of bloodstream infections. Phases 1 and 2 included febrile adult patients and were performed during Malawi's wet and dry seasons. Infection patterns varied significantly by this parameter. Phase 3 included all children hospitalized during the field period.

South Africa. Transferred to South Africa current protocols for amplification and sequencing of HIV-1 for drug-resistance mutations.

Thailand.

Sequenced and identified potential drug-resistance mutations in subtype E infected infants receiving AZT treatment.

Determined optimal method for quantifying HIV RNA in plasma of clade B' and E.

Thailand/Côte d'Ivoire. Continued to sequence and analyze discordant samples identified by subtype-specific peptide and probe assays at international field stations.

Uganda. Completed evaluation of subtype A and D -specific peptide technology for Uganda, and transferred such technology. A manuscript describing evaluation is in preparation.

UNAIDS/WHO. Initiated collaborative study with UNAIDS/WHO for evaluating genotyping technology as part of an HIV-1 drug-resistance surveillance program in Latin America.

1999 Plans

Immunobiology of HIV Infection

HIV coreceptor expression. Continue studies to examine the expression of HIV coreceptors, CXCR4 and CCR5, and markers of activation (CD38) on lymphocyte subpopulations in HIV-positive and -negative individuals.

Transmission of HIV from mothers to infants. In collaboration with DHAP-SE/NCHSTP, examine the usefulness of activation (CD38), immune responsiveness (CD28), chemokine receptors (CXCR4, CCR5), and maturation markers (CD45RA, CD45RO) to predict perinatal HIV transmission.

Quantitative CD38 expression. Evaluate the performance of CD38 quantitation in HIV infection.

HIV infection in vitro. Determine characteristics of cells that become infected with HIV in vitro, including the expression of chemokine receptors and maturation markers on p24+ T cells.

HIV clinical course.

Complete data collection and analyses of the pilot phase of the HIP/VAMC collaborative study to longitudinally assess the relationships among intracellular cytokine patterns, HIV viral titers, CD4 cell counts, and other immune markers and the clinical course of HIV infection both before and during antiretroviral therapy.

Fully implement the VAMC study to include a longitudinal assessment of patients over a 3-year period. Blood studies will be conducted a) every 4-6 months; b) at times of acute symptomatology; and c) with changes in antiretroviral therapy (funding source undetermined).

Determine the relationships among stage of HIV infection (viral titers and CD4 counts), immune parameters (including intracellular cytokines), and nutritional status (serum vitamin A, vitamin E, and iron) in hospitalized Malawian children.

HIV infection in vitro. Submit manuscripts concerning the effects of HIV infection and antigens and the modulatory effects of anti-CD3 and mitogens on CD3, CD4, and CD8.

HIV infection in vitro. Examine the intracellular cytokine profiles of various cell populations within this *in vitro* system, including p24+, CD4+, and CD8+ lymphocytes.

CD4 T-cell turnover. Complete further analysis of CD4 recovery and activation markers after effective antiretroviral therapy.

HIV entry mechanisms. Continue studies of HIV entry mechanisms and the relationship of viral structures, chemokine receptor expression, and subcellular localization.

T-cell activation and viral load. Publish results of a serial study of viral load determinations and immune-activating events in 18 individuals clinically followed for 26 weeks to assess the effect of immune-activating events on viral load.

Needlestick study. Continue collaboration with investigators in the HIV Infection Branch, HIP, in a multicenter study of HCWs exposed to HIV to determine whether any immunologic response to HIV occurs following such exposures. Coordinate laboratory testing and data analysis for this study. Evaluate the influence of hepatitis C virus transmission in this setting on the risks for HIV transmission.

ICL. Continue investigation of ICL to further characterize this "experiment of nature" both to impact the clinical care of patients with this condition as well as to compare ICL and HIV infection to increase our knowledge of the specific immunologic

defects found in HIV infection.

Simian foamy virus (SFV). In collaboration with the HIV/AIDS and Retrovirology Branch, DASTLR, assist in the evaluation of individuals infected with SFV and other simian retroviruses by looking for changes in their immune systems and their immunologic responses to these viruses. Develop an *in vitro* test to assess the immune response to such agents.

HIV long-term nonprogressors. Begin study of HIV long-term nonprogressors to determine either host or viral factors involved in their disease status. The study will be conducted in collaboration with investigators in the HIV/AIDS and Retrovirology Branch, DASTLR.

HHV-8

Continue investigations into the immunology of HHV-8 infection and its association with Kaposi's sarcoma and other diseases.

Evaluate serologic and PCR-based assays for the diagnosis of HHV-8 infection.

Participate in the prospective longitudinal study of individuals with HHV-8 infection in collaboration with the Viral Exanthems and Herpesvirus Branch, DVRD/NCID.

Evaluate risk factors for developing HHV-8 infection in men who have sex with men to determine routes of transmission.

Complete the analysis and report results of a retrospective study of infection with HHV-8 and the course of the infection in a cohort of HIV-infected men who have sex with men in Atlanta.

Continue to assist in the development of a peptide-based immunoassay for the detection of HHV-8/Kaposi's sarcoma herpesvirus antibodies in human sera/plasma.

HIV Immune Response

HIV superantigen. Publish manuscript examining the HIV LTR region known to be homologous to a murine SAG and a highly T-cell immunogenic region of the HIV envelope region for potential SAG activity.

γδ T-cell subsets. Publish manuscript examining the immune parameters, and especially T- cell subsets, in *Mycobacterium tuberculosis* and non-typhus salmonella bloodstream infections.

HIV infection, cytokines, and malaria. Examine the relationship among malaria parasitemia, stage of HIV infection, and immune parameters (including cytokines) in participants of the Malawian study.

Immune activation. Evaluate the characteristics of Malawi participants who exhibited spontaneous cytokine production.

Natural killer cells and cytokine production. Evaluate the characteristics of participants in the Malawian study who have relatively high proportions of natural killer cells and/or CD3+CD16/56+ lymphocytes. Characterize these cell populations more fully, especially concerning cytokine production.

Cytotoxic T-cell studies in HIV-infected, HIV-exposed uninfected, and transiently infected persons. Continue studies of Thai and Abjidan HIV-positive and HEPS individuals and of domestic HCWs; publish data collected from 1997-1998.

HIV vaccine studies. Publish manuscripts from other murine studies; collaborate with and initiate macaque DNA HIV vaccine experiments at Yerkes and CDC.

Cytokine mRNA studies. Publish findings on cytokine dysregulation in TOS/EMS in vitro studies.

HTLV studies. Publish manuscript describing findings related to HTLV and autoimmunity.

CD4 antiviral constructs. Improve expression system. Assess immunopotentiating capacity of CD4/C3d chimeric construct in a mouse immunization protocol.

IgA- and IgG-specific anti-HIV response. Continue studies of isotype-specific immune responses in perinatally infected infants, and determine relationship to results with the detuned assay.

HIV Drug-Resistance Studies

Viral fitness and drug resistance. Examine biological replication kinetics of resistant isolates from patients on antiretroviral therapywho differ in apparent CD4 T-cell recovery.

Resistence genotyping. In collaboration with the HIV and Retrovirology Branch, HIP, and DHAP-SE, continue to sequence HIV-1 infected individuals as part of the ongoing drug- resistance surveillance program in the United States.

Genotyping technology. Make Visible Genetics automated sequencing technology available online for use in U.S. surveillance programs and international studies.

HIV Diagnostics and Evaluation

Multiplex cytokine assessment. Continue support through this fiscal year for the SRP collaborative project. When the multiplex assay has been optimized, use Malawi samples to compare results obtained from this technique to those obtained using the same monoclonal/polyclonal reagents and an ELISA plate technique.

Multiplex PCR for diagnosis of infection. Assist in studies using PCR to determine various causes of bloodstream infections, including studies on the relationship between mycobacterial culture results and PCR and detection of organisms not found by blood culture.

PCR technology improvement. Development a technique for HLA genotyping of specimens with low amounts of DNA.

Seroincidence. Select most promising assays that distinguish HIV seroincident cases from established infection for larger analysis, with the ultimate goal of identifying an assay with high throughput potential for assessing seroincidence in large populations with samples from a single time point (cross-sectionally).

AF-CDC-WHO antinuclear antibody reference lab. Continue reference diagnostic and standardization activities. Identify and prepare a PM/SCL70 reference serum.

Evaluate and implement the detuned assay. Assay HIV seroincident specimens from the United States, Thailand, and Uganda to validate the calibration. The detuned assay will be used in populations with unknown HIV incidence.

Training on detuned assay. Train personnel from additional sites, including sites in Thailand, Côte d'Ivoire, and Uganda, on the use of the detuned assay.

Vaccine studies. Test Phase II trial specimens from VaxGen using promising peptide-based tests as a potential tool for discriminating between vaccinated and HIV-infected individuals.

Immunogenetics

Genetic studies.

Publish the new finding on IL-6 SNP.

Publish new finding on recombinant break point in the multicase arthritis family.

Continue studies of African Americans with RA

Continue to apply HLA algorithms to understanding HLA associations with HIV outcomes in the MACS, and publish relevant findings.

Conduct studies on HLA and RA/hepatitis B vaccinees and susceptibility to TB and leptospirosis.

HERS. Complete HLA and chemokine receptor analysis of 1100 specimens from the DHAP-SE/NCHSTP HIV Epidemiology Research Study (HERS).

Human genes and HIV infection. Continue genetic studies concerning the natural history of HIV.

Tuberculosis. In collaboration with NCHSTP, perform NAT gene analysis on a population with tuberculosis.

International

Côte d'Ivoire. In collaboration with the International Activity, DHAP-SE/NCHSTP, determine IgA- and IgG-specific anti-HIV responses in breast milk from infected mothers and the relationship to perinatal HIV transmission.

Malawi. Complete all analyses and discuss, in person, with collaborators in Malawi the results and implication of all findings. Depending upon the results of these analyses, this study may or may not be expanded to other Asian and African countries.

South Africa. In collaboration with the International Activity, DHAP-SE/NCHSTP, assist South African collaborators with peptide-specific subtype and HIV-1 drug-resistance mutation surveillance by transferring current technologies for evaluation.

Thailand.

Perform additional CCR5 sequence analysis on CONRAD population, and perform CCR5 analysis in the Bangkok study.

Determine resistance genotype in mothers and their infants from the Thai AZT trial. Determine genotypic and phenotypic patterns for HIV-1-infected Thai infants and adults receiving therapy with other (non-AZT) HIV drugs.

Using detuned assays, evaluate specimens from the perinatal I study to identify recent seroconverting mothers and the relationship of maternal recent infection to perinatal transmission.

In collaboration with HAC, evaluate various HIV-1 immune and viral parameters in infants to determine their relationship to transmission and disease progression.

Thailand/Côte d'Ivoire. In collaboration with the International Activity, DHAP-SE/NCHSTP, continue development and evaluation of HIV-1 drug-resistance mutation technologies in non-U.S. B subtypes, with focus in Thailand and Côte d'Ivoire.

Uganda. Transfer subtype A- and D-specific peptide technology to Uganda for evaluation.

Uganda/Thailand/Côte d'Ivoire. Continue support for the International Activity, DHAP-SE/NCHSTP, by analyzing discordant samples identified at field stations using peptide and probe subtyping technology.

UNAIDS/WHO -Evaluate UNAIDS/WHO quality-assurance panel for HIV-1 drug resistance.